

What is claimed is:

1. A method of assaying for protease activity, comprising:

providing a nucleic acid construct having a sequence encoding an amino terminal portion of a fluorescent reporter fused to a sequence encoding a substrate of a protease followed by a sequence encoding a carboxy terminal portion of a fluorescent reporter protein;

expressing a recombinant fluorescent substrate in the presence of the protease;

detecting a change in quenching of fluorescence in the recombinant fluorescent substrate as an indication of protease activity.

2. The method of claim 1 wherein the presence of a peptide bond between the amino and carboxyl-terminal fragment of the fluorescent substrate is essential to generate or maintain fluorescence.

3. The method of claim 1 wherein fluorescence is quenched by cleavage in the protease substrate sequence.

4. The method of claim 1 wherein the intrinsically fluorescent protein is GFP.

5. The method of claim 1 wherein the protease is introduced by expression from a nucleic acid construct.

6. A method for identifying a protease that cleaves a target amino acid sequence, comprising:

providing a nucleic acid construct having a sequence encoding an amino terminal portion of a fluorescent reporter fused to a sequence encoding a desired substrate target followed by a sequence encoding a carboxy terminal portion of a fluorescent reporter protein;

expressing of the recombinant fluorescent substrate in the presence of a plurality of proteases;

detecting at least one of the plurality of proteases that recognize the target sequence by quenching of the fluorescence of the reporter.

7. The method of claim 6 wherein the fluorescent reporter protein is GFP.
8. A method for determining a substrate recognized by a test protease, comprising:
inserting each of a plurality of fusion nucleic acid sequences encoding a plurality of
protease substrate sequences between sequences encoding the amino and carboxyl-
terminus of an intrinsically fluorescent protein to form a library of fusion nucleic
acids;
expressing the library of fusion nucleic acids to generate a library of recombinant fusion
proteins in the presence of the test protease; and
identifying members of the recombinant fusion protein library having quenched
fluorescence.
9. The method of claim 8 wherein the fluorescent reporter protein is GFP.
10. A method of assaying proteolytic activity between a protease and a protease substrate
sequence of amino acids comprising:
(a) inserting a nucleic acid sequence of amino acids into a surface exposed loop of an
intrinsically fluorescent protein to form a recombinant protein;
(b) expressing the recombinant protein substrate;
(c) purifying the recombinant protein substrate; and
(d) detecting quenching of fluorescence in the presence of a protease.
11. The method of claim 10 wherein the fluorescent protein is GFP.
12. A method of assaying proteolytic activity between a protease and a protease substrate
sequence of amino acids, comprising:
(a) inserting the protease substrate sequence of amino acids into a surface exposed loop of
an intrinsically fluorescent protein to form a recombinant protein;
(b) detecting quenching of fluorescence in the presence of the protease.

13. The method of claim 12 wherein the intrinsically fluorescent protein is GFP.